The functional significance for the mobility of loop regions of GM2AP was probed using crosslinking agents to tether the two mobile loops via disulfide crosslinking to cysteine residues which were incorporated into each of the mobile loops of GM2AP. The tethered GM2AP constructs were functionally evaluated using fluorescence spectroscopy. Danysl-DHPE a fluorescently labeled lipid substrate was utilized to analyze the ability of the crosslinked GM2AP to extract the fluorescent lipid from large unilamellar vesicles containing POPC:dansyl DHPE. A blue shift in the wavelength of maximum emission for the dansyl-DHPE extracted by GM2AP allowed the amount and kinetics of lipid extraction to be assayed. The tethered constructs were also assayed for their ability to bind and sediment with a lipid membrane. Results indicate that tethering of the mobile loops mitigate the ability of GM2AP to extract its lipid ligand substrate.

292-Pos

Intact Protein Component of Cytochrome bc1 Complex Is Not Essential For the Superoxide Generation

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In addition to its main functions of the electron transfer and proton translocation, the cytochrome bc1 complex also catalyzes generation of superoxide upon oxidation of ubiquinol in the presence of molecular oxygen. The mechanism of superoxide generation by bc1 remains elusive. The superoxide generating activity seems to inversely proportional to the electron transfer activity. Complexes with less complexity in subunit structure tend to have higher superoxide generating activity. The maximum superoxide generating activity is observed when the complex is inhibited by antimycin. When the complex is treated with proteinase K, the electron transfer activity decreased and the superoxide generating activity increased as the incubating time increased. The maximum activity is obtained when the protein components of the complex is completely digested, indicating that intact proteins play little role in superoxide generation. It is speculated that the hydrophobic environment and the availability of a high potential electron acceptor from the complex is responsible for the activity. This speculation is confirmed by the detection of superoxide formation upon oxidation of ubiquinol by a high potential oxidant such as cytochrome c or ferricyanide in the presence of phospholipid vesicles or micellar solution of detergents. Little superoxide formation was observed when ubiquinol is oxidized under the hydrophilic conditions. This work was supported in part by a grant from NIH (GM30721).

293-Pos

Alteration of Membrane Protein Function Through the Photo-Activation of the Hydrophobic Probe Iodonaphtylazide

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Iodonaphtylazide (INA) has been developed 30 years ago to determine the penetration of proteins into biological membranes. Due to its very high partition coefficient into lipidic bilayers and the sensitivity of its detection, ^{125}INA has long been used to label and identify membrane proteins, to study membrane dynamics and fusion and to detect protein-membrane interactions. The labeling of membrane proteins is mediated by the azido moiety of INA that can be activated by near UV light. Upon excitation, a nitrene radical is formed leading to the covalent binding of membrane proteins in the surroundings. Besides labeling, this binding results in specific alterations of the hydrophobic domains of proteins. When applied to enveloped viruses, the treatment resulted in a complete loss of infectivity. While the overall integrity of the virus is preserved, the ability of the viral envelope glycoprotein to promote full fusion is impaired. In the case of influenza, hemifusion was not affected by the treatment indicating a blockage at the late stage of fusion. We also tested the effect of hydrophobic labeling on the function of cellular transmembrane receptors. The lateral mobility of chemokine receptors, which are G coupled receptors, was reduced and CXCR4 lost its ability to signal in response to external stimuli. However, the activity of a tyrosine kinase receptor (IGF1) was increased. The activity of a multi drug resistance transporter MRP1 was blocked by the hydrophobic treatment. Overall, photo-activation of INA in various cell lines, including those over-expressing the multi-drug resistance transporters MRP1 or Pgp, leads to apoptosis.

294-Pos

Simultaneous Measurement of Phagosome and Plasma Membrane Potentials in Human Neutrophils By Di-8-Anepps and SEER

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Neutrophils are the first line of defense against invading bacteria. Neutrophils engulf the invaders into an internal vacuole, the phagosome. Accompanying phagocytosis is the respiratory burst, in which NADPH oxidase produces reactive oxygen species by transporting electrons from cytosolic NADPH across the membrane to either intra-phagosomal or extracellular oxygen. It is well known that the plasma membrane depolarizes during the respiratory burst but very little is known about the membrane potential of the phagosome. Here we monitor the membrane potential of phagosomes as well as the plasma membrane during the phagocytosis of opsonized zymosan.
Neutrophils were isolated from whole blood and plated on glass coverslips. The cells were loaded with 5 μM di-8-ANEPPS for 30 minutes before excess dye was washed away. The cells were stimulated by addition of 2 mg/ml serum opsonized zymozan (OPZ) and were visualized using a Leica SP2 confocal microscope. SEER imaging was performed by simultaneously acquiring two images at 488 nm and 545 nm and collecting at emission ranges 470-560 nm and 570-700 nm respectively.The neutrophil plasma membrane depolarized rapidly coinciding with phagocytosis of the first OPZ particle. The potential generally decreased somewhat, but the plasma membrane potential generally remained positive to 0 mV for many minutes, during which time several phagocytotic events were typically observed. DPI produced repolarization, confirming that the depolarization was due to the electrogenic activity of NADPH oxidase. The membrane potential of each phagosome was highest upon formation and decreased within several minutes, often falling to negative voltages, while the plasma membrane remained depolarized. The phagosome membrane potentials were independent of, and at their peak often exceeded the plasma membrane potential. This study is the first to monitor the phagosome membrane potential in living cells.

295-Pos

Flio Is Not Required For Motility in *Salmonella* If Its Cytoplasmic Domain and Flip Mutant Suppressors Are Expressed

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The propeller-like flagella found in bacteria, and used for motility, possess a specialized secretion apparatus, which is imbedded in the cell membrane for their formation. Its components are highly conserved not just amongst flagellar systems, but also to the Type III secretion apparatus used by some bacteria in conjunction with virulence-associated needle complexes. The flagellar secretion system of *Salmonella typhimurium* consists of 6 integral membrane proteins: FlhA, FlhB, FliO, FliP, FliQ, and FliR. However, for the virulence-associated needle complexes of *S. typhimurium* and the flagellum of the bacterium *Aquifex aeolicus* a homolog of FliO is apparently absent. In this study we showed that deleting the *fliO* gene from the chromosome of a motile strain of *Salmonella*resulted in a drastic decrease of motility. However, incubation of the *fliO* mutant strain in motility agar, gave rise to mutants containing suppressors that help to restore partial motility. One class of the suppressor mutation was found in the *fliP* gene. Using truncation and site-directed mutagenesis analysis of the FliO protein, it was shown that expression of FliO cytoplasmic domain in cells with the *fliO* gene deleted can also partially restore the motility. When the FliO cytoplasmic domain was expressed in the FliP suppressor mutant strains an additive effect was observed, and near wild-type levels of motility were regained. The FliO cytoplasmic domain was purified and studied using circular dichroism spectroscopy. Based on secondary structure prediction it should contain beta-structure and alpha-helices, however, we showed that this domain is disordered and its structure is a mixture of beta-sheet and random coil. We assume that the FliO cytoplasmic domain becomes structured while interacting with its binding partners.

296-Pos

Surface Activity of Surfactant Protein SP-B and SP-C in Different Lipid Environments

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Pulmonary surfactant is a mixture of lipids and proteins, essential to reduce the surface tension at the air-liquid interface in the alveoli of mammalian lungs and so stabilizing the respiratory surface. Lack of an operative surfactant is associated with severe respiratory pathologies and supplementation with exogenous surfactants has been widely approached as a potential therapeutic intervention. However, the optimal lipid and protein composition of exogenous surfactants has not been properly established, and clinical surfactants currently in use differ substantially in terms of their lipid and protein moieties. In the present study we have compared the surface activity of native SP-B and /or SP-C, purified from porcine lungs, in the Captive Bubble Surfactometer (CBS), once reconstituted into two different synthetic lipid mixtures: DPPC/POPC/POPG/Chol (50:25:15:10) (lipidS), a mixture mimicking lipid composition in natural